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Volkensinin: A New Limonoid from *Melia volkensii*

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Abstract: Bioactivity-directed fractionation of the root bark of *M. volkensii* Gürke (Meliaceae) resulted in the isolation of a new compound: volkensinin (**1**). The structure of **1**, which contains a unique 5-membered C ring, has been elucidated by the analysis of spectral data. Compound **1** showed weak cytotoxicities against six human tumor cell lines.

Limonoids from *Melia* have attracted much attention because of their wide range of biological activities, including insect antifeedant and growth regulating properties, a variety of medicinal effects in animals and humans, as well as antifungal, bactericidal, and antiviral activity.[1] As part of our continuing search for antitumor and pesticidal agents from higher plants, we have investigated the root bark of *Melia volkensii* Gurke (Meliaceae) obtained from Kenya. We report herein the structure of one of the bioactive compounds which we have found to be a new type of limonoid having a unique five-membered C ring. The formation of this new skeletal type is of biogenetic interest.

The isolation of volkensinin (**1**) was guided by the brine shrimp lethality test (BST)[2] using repetitive open column and HPLC chromatography to separate the partitioned ethanol extract.[3-5] The elemental formula, $C_{32}H_{42}O_{11}$, was determined by high-resolution mass measurement (HRMS) of its molecular ion at *m/z* 603.2805 (calcd 603.2811). The ^{1}H spectrum indicated a furan ring, three acetates, two hydroxyl groups, and a skeleton similar to that of salannin (**2**)[6] which belongs to a group of limonoids with opened or rearranged C rings. However, unlike most of the compounds in this group which contain four tertiary methyl groups, only three were seen in the ^{1}H-NMR of **1**, at δ 0.98 (H-19), 1.26 (H-29) and 1.32 (H-18). The positions of these three methyl groups were assigned by analysis of the HMBC spectrum (Table 1). There was no normal tertiary methyl signal for H-30, hence, H-30 was presumed to have reacted in some unusual way.

Carefully examining the COSY spectrum, we found that the oxymethine proton at δ 4.41 (H-12) in **1** showed coupling with not only one of the methylene protons at δ 1.72 (H-11), but also with one proton of another methylene group at δ 1.80, and the latter was not further coupled. This observation suggested that ring C had opened and C-12 had recyclized onto C-30. The correlations between C-8 and H-30, as well as the correlation between C-8 and H-12 in the HMBC spectrum further supported this conclusion.

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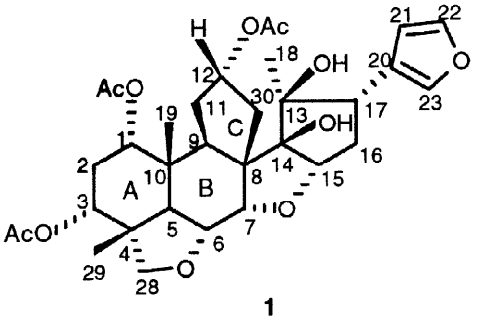

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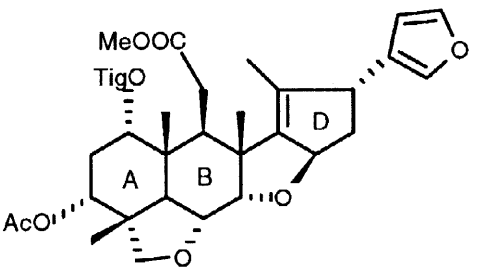

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Compared with **2**, no double bond signals, except those arising from the furan ring, were evident in the ^{13}C-NMR spectrum of **1**. Instead, two quarternary carbons at δ 96.06 and 80.53 were observed. The locations of the chemical shifts of these two carbons suggested that they were oxygenated, and the HMBC spectrum allowed us to assign them as C-14 and C-13, respectively (Table 1). Two hydroxy groups were present in **1** as indicated by the disappearances of the resonance peaks at δ 3.10 and 2.65 on addition of D_2O in the ^{1}H-NMR spectrum. Attempted acetylation of **1** with acetic anhydride-pyridine failed to give products, and the two hydroxy groups were, therefore, assumed to be connected with quarternary carbons and were placed on C-13 and C-14. These assignments were supported by a small cross peak between δ 3.10 (C_{13}-OH) and 1.32 (H-18) shown in the COSY spectrum.

Comparison of the ^{1}H- and ^{13}C-NMR data of **1** with those of **2** revealed the presence of the 6,28-oxide ring and allowed us to place two of three acetate groups at C-1 and C-3. These moieties along with the two hydroxyls and a furan ring accounted for ten of the total of eleven oxygens in **1**. Since no additional carbonyl or hydroxyl signals were evident in the ^{1}H- and ^{13}C-NMR spectra, the remaining oxygen was, therefore, assumed to be in an ether linkage. This ether linkage could be between C-7 and C-12, C-12 and C-15, or C-7 and C-15. In the COSY spectrum of **1**, H-7 showed correlation with H-6 and nothing else, and H-15 showed correlation with two methylene protons of H-16 and nothing else; thus, neither the connection of C-7 with C-12 nor the connection of C-12 with C-15 was possible. Consequently, this oxide was deduced to be 7,15-oxide, and the third acetate group was, therefore, placed on C-12.

The relative stereochemistry of **1** was established from extensive examination of the NOESY spectrum. The orientation of H-1β, H-3β, H-5α, H-6β, H-7β, H-19β, H-28α and H-29β were determined to be the same as those in typical limonoids. Although all the reported limonoids with 7,15-oxides have the H-15α orientation, H-15 in **1** was assigned to be β based on the observation of the cross peak between H-15 and one of the H-30 protons. The most important correlations in the NOESY spectrum are summarized in Table 2.

Table 1. ^{1}H- and ^{13}C-NMR Data for **1**.

positions	δ C* (mult.)	δ H	mult.	coupling (Hz)	HMBC correlations
1	71.96 (d)	4.70	t	3.0	H-3, H-19
2	28,08 (t)	2.12	m		
3	71.42 (d)	4.91	t	3.0	H-1, H-29
4	42.66 (s)				H-5, H-29
5	38.96 (d)	2.70	d	12.5	H-1, H-7, H-19, H-28, H-29
6	74.29 (d)	3.96	dd	4.0, 12.5	H-5, H-7, H-28
7	67.54 (d)	4.56	d	4.0	H-5
8	60.50 (s)				H-12
9	35.44 (d)	3.09	dd	5.0, 9.5	H-12
10	38.96 (s)				H-19
11	33.58 (t)	1.62	m	2.0, 5.0, 14.0	
		1.72	ddd		
12	76.30 (d)	4.41	bs		H-30
13	81.15 (s)				H-12, H-15, H-16, H-18, H-30
14	96.06 (s)				H-15, H-16, H-17, H-18, H-30
15	80.55 (d)	5.24	t	7.5	
16	32.19 (t)	1.55	dt	7.0, 12.5	H-17
		2.43	dt	7.5, 12.5	
17	45.05 (d)	2.89	dd	7.0, 12.5	H-18
18	20.46 (q)	1.32	s		
19	16.55 (q)	0.98	s		
20	123.4 (s)				H-17, H-21, H-22, H-23
21	139.5 (d)	7.20	m		H-23
22	111.0 (d)	6.40	dd	0.5, 1.5	H-21, H-23
23	142.4 (d)	7.36	t	1.5	H-21, H-22
28	78.73 (t)	3.62	d	7.0	H-5, H- 29
		3.65	d	7.0	
29	20.96 (q)	1.26	s		H-5, H-28
30	42.2 7 (t)	1.56	d	10.5	
		1.80	dd	2.0, 10.5	
C=O	170.4 (s)				CH_3 (δ 2.09)
CH_3	21. 76 (q)	2.09	s		
C=O	170.3 (s)				CH_3 (δ 2.02)
CH_3	21 .54 (q)	2.02	s		
C=O	1 69.6 (s)				CH_3 (δ 2.12)
CH_3	20.96 (q)	2.12	s		

*The assignments were made by DEPT, COSY, HMQC (J = 140 Hz) and HMBC (J = 10 Hz).

Table 2. Selective NOESY correlations of **1**.

H #	correlations to H #	H #	correlations to H #
1	H-2β, H-19	**7**	H-6, H-30α, H-30β
3	H-2β, H-29	**9**	H-5, H-18
5	H-28	**12**	H-11, H-30α, H-30β
6	H-7, H-19, H-29, H-30β	**15**	H-16α, H-17, H-30α

Compound **1** represents a unique structural type in the group of limonoids with opened or rearranged C rings. This is a large and important group containing some very complex compounds, such as the insecticide,

azadirachtin.[7] The biogenesis of this group of limonoids depends on the opening of ring C by hydrolysis.[8] Upon its opening, the rotation of the D ring about the C-8, C-14 bond and the closure to C-15 may occur, giving limonoids having seven-membered C rings and β-substituted furan rings such as in heudebolin.[9] The opening of ring C could also provide extra flexibility for the formation of a 7,15-oxide and create another common type of limonoids in this group, such as **2**. Although most of the reported limonoids with 7,15-oxide rings have H-7β, H-15β stereochemistry and their C rings remain open, compound **1** contains a H-7β, H-15β orientation and a closed C ring. The formation of this five-membered ring C is very unusual, and its biosynthetic origin remains to be determined.

Compound **1** showed weak bioactivity in the BST[2] (LC_{50} 57 μg/mL), and it was generally but weakly cytotoxic against six human tumor cell lines[10] [ED_{50} 27.90, 28.35, 33.56, 29.55, 8.43, and 28.51 μg/mL in A-498, PC-3, PACA-2, A-549, MCF-7, and HT-29, respectively]. Adriamycin, as a positive control in the same run, gave ED_{50} values of 4.24×10^{-3}, 2.76×10^{-2}, 1.33×10^{-2}, 4.47×10^{-3}, 9.19×10^{-2}, and 2.79×10^{-2} μg/mL, respectively.

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5 **1**: 8 mg was isolated; mp 130 -135 °C; $[\alpha]^{25}_{D}$ = -100°; IR (film on KBr plate) 3521, 2975, 2895, 1731, 1375, 1250 cm^{-1}; UV (in MeOH) γ_{max} = 204 nm (log ε = 6.32).
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10 The 7-day MTT *in vitro* cytotoxicity tests followed the standard protocols and were performed at the Cell Culture Laboratory, Purdue Cancer Center.